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ORIGINAL ARTICLE

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Identification of expression profiles of sorghum genes in response to greenbug phloem-feeding using cDNA subtraction and microarray analysis

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Abstract The phloem-feeding by greenbug (*Schizaphis graminum*) elicits unique interactions with their host plants. To investigate the expression profiles of sorghum genes responsive to greenbug feeding, two subtractive cDNA libraries were constructed through different combinatorial subtractions in a strong greenbug resistance sorghum M627 line and a susceptible Tx7000 line with or without greenbug infestation. A total of 3,508 cDNAs were selected from the two cDNA libraries, and subsequent cDNA microarray and northern blot analyses were performed for identification of sorghum genes responsive to greenbugs. In total, 157 sorghum transcripts were identified to be differentially expressed by greenbug feeding. The greenbug responsive genes were isolated and classified into nine categories according to the functional roles in plant metabolic pathways, such as defense, signal transduction, cell wall fortification, oxidative burst/stress, photosynthesis, development, cell maintenance, abiotic stress, and unknown function. Overall, the profiles of sorghum genes, responsive to greenbug phloem-feeding shared common identities with other expression profiles known to be elicited by diverse stresses, including pathogenesis, abiotic stress, and wounding. In addition to well-known defense related regulators such as salicylic acid, jasmonic acid, and abscisic acid, auxin and gibberellic acid were also involved in mediation of the defense responses against greenbug phloem-feeding in sorghum.

Keywords cDNA subtraction · Microarray analysis · Molecular defense mechanisms · Plant–aphid interactions · *Schizaphis graminum* · *Sorghum bicolor*

Abbreviations ABA: Abscisic acid · ET: Ethylene · GA: Gibberellic acid · JA: Jasmonic acid · ROS: Reactive oxygen species · SA: Salicylic acid

Introduction

The aphid greenbug, *Schizaphis graminum* (Rondani), has been reported as one of the serious threats to staple crops, including sorghum (*Sorghum bicolor*) (Stone et al. 2000). Greenbug damage causes tremendous economic losses in sorghum production to the amount of approximately $21.3 million annually in Texas alone (Katsar et al. 2002). In addition, aphids transmit more than 275 viruses in a non-persistent manner via salivation during intercellular phloem-feeding (Powell 2005). The greenbug is a typical phloem-feeder, which uptakes photoassimilates and other liquid substances mainly from phloem sieve elements in plants (Miles 1999). A detailed understanding of molecular defense mechanisms against aphid phloem-feeding in sorghum will help to develop durably resistant sorghum cultivars against aphids.

Due to their sessility, plants cannot avoid the surrounding threats actively. Instead, plants operate elaborate defense systems against diverse biotic and abiotic stresses by orchestration of signal pathways, leading to the activation of versatile defense responses. The crosstalk between signal pathways elicited by molecular regulators in plants has been widely issued. To defend against the numerous types of challenges, plants develop efficacious defense systems via the crosstalk amongst

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endogenous signal molecules such as salicylic acid (SA), jasmonic acid (JA), ethylene (ET), nitric oxide (NO), and reactive oxygen species (ROS) (Reymond and Farmer 1998). For instance, antagonistic relationship was observed between SA dependent resistance on pathogenesis and JA dependent resistance on insect feeding in tobacco plants (Schenk et al. 2000). In several studies, SA suppressed JA and ET dependent signal pathways and vice versa (Dmitriev 2003). Analysis of promoter sequence regions in cytochrome P450 genes, which responded to either biotic-, abiotic-stress, or both stresses, verified that the promoter regions contain common regulatory motifs (Narusaka et al. 2004).

Compared to the extensive progress in molecular biological understanding of plant defense mechanisms in response to pathogen attack, molecular interpretation of plant defense responses against insect feeding has been much less accomplished (Kessler and Baldwin 2002). The plant defense responses against insect herbivory are known to be controlled by multiple molecular regulators, including JA, SA, ET, and ROS (Walling 2000). The plant hormone SA plays a crucial role in the expression of defense genes responding to pathogen attack (Hammond-Kosack and Jones 1996). Accumulation of SA in plants elicits local hypersensitive responses (HR) and systemic acquired resistance (SAR) (Maleck and Dietrich 1999). JA is known to conduct direct defense responses, including synthesis of toxic compounds to insects, against herbivores in plants (Stotz et al. 1999; Turner et al. 2002). Ryan (2000) found that systemin released from wounding sites by insect feeding invoked elicitation of signal cascades for production of JA via the octadecanoid pathway. ET plays a pivotal role in plant development and growth (Ecker 1995). Inhibition of ET biosynthesis resulted in a significant reduction ($<30\%$) of JA accumulation in wounding sites (Wang et al. 2002). JA and ET showed a synergistic relationship in the production of proteinase inhibitors and defensins in Arabidopsis (Penninckx et al. 1998). The crosstalk between molecular regulators is a complex process that shows versatile correlations. Silencing gene expression of tobacco phenylalanine ammonia lyase gene (PAL) catalyzing initial step of the phenylpropanoid biosynthesis weakened accumulation of endogenous SA in concurrence with an increment of JA biosynthesis (Felton et al. 1999). SA inhibited an enzymatic action of 13*S*-hydroperoxide dehydrogenase, leading to a blockage of conversion from 13*S*-hydroperoxylinolenic acid to 12-oxo-phytodienoic acid (OPDA), which is a precursor of JA biosynthesis (Pena-Cortes et al. 1993). Inhibition of proteinase inhibitors elicited by JA and methyl-JA (MeJA) occurred by SA and acetyl-SA treatment (Doares et al. 1995). During insect herbivory, ROS was produced and played an important role in signaling, by acting as intercellular messengers (Reymond and Farmer 1998; Walling 2000). Activation of NADPH oxidase by wounding results in bursting of ROS, including hydrogen peroxide, and hydrogen peroxide accumulation induces subsequent biosynthesis of JA, leading to the expression of defense genes against insect feeding (Orozco-Cardenas et al. 2001; Turner et al. 2002). Plants utilize blends of volatiles comprising terpenes and fatty acid derivatives in response to insect feeding (Pichersky and Gershenzon 2002). The volatiles serve as detergents to herbivores, attractants to natural enemies of herbivores, and messengers to neighboring plants (Pare and Tumlinson 1999).

Aphids occupy about half of the insect biotypes on cultivated crops (Shufran et al. 2000). Nevertheless, little is known about the molecular responses to aphid phloem-feeding in plants. Unlike chewing herbivory that produces extensive damage on plant tissues, aphids cause minor injury while feeding. Thus, plants recognize greenbug feeding as a pathogenic infection, and sequential defense responses are enforced via signal cascades elicited by SA, JA, and ET (Walling 2000). In Arabidopsis, an analysis of expression profiling in response to aphid phloem-feeding suggested that arrays of genes induced by oxidative stress, calcium-dependent signals, and pathogenesis were prevalent in the profiles (Moran et al. 2002). It has been known that plant defense responses against insect feeding are not only induced by tissue damages but also by insect saliva and regurgitants (Miles 1999; Halitschke et al. 2001). The saliva of greenbugs contains non-enzymatic reducing compounds, lipase, oxidases, and enzymes depolymerizing polysaccharides, such as pectinase and cellulase (Miles 1999). Zhu-Salzman et al. (2004) demonstrated that greenbug feeding on sorghum activated JA- and SA-regulated genes, likely linked to the host defense responses. Normal allocation of carbon and nitrogen in alfalfa was disrupted by aphid feeding and subsequent morphological modifications followed (Girousse et al. 2005). Expression profiling of sorghum genes associated with treatments by MeJA, SA, and aminocyclopropane carboxylic acid demonstrated that both synergistic and antagonistic effects were appeared in the expression of genes induced by SA or MeJA (Salzman et al. 2005).

This study pursued further understanding of sorghum molecular defense mechanisms in response to greenbug phloem-feeding. Using two different sorghum lines, M627 (resistant) and Tx7000 (susceptible), two subtractive cDNA libraries were constructed. The subsequent cDNA microarray analyses were performed based on the subtracted cDNA clones. Then, northern-blot analyses were employed to confirm the data obtained from the microarray analyses. Sorghum genes that showed differential expression levels in response to greenbug feeding were identified by database searches, and then classified into functional categories. The results of this study suggest that the defense responses against greenbug phloem-feeding in sorghum are coordinately modulated by versatile molecular regulators such as SA, JA, ROS, ABA, GA, and auxin. It is also suggested that greenbug phloem-feeding is accompanied by multiplex stresses similar to wounding, drought, oxidative stress, pathogenesis, water stress, and insect herbivory.

Materials and methods

Plant growth and aphid culture conditions

Seeds from the two different sorghum (*S. bicolor*) lines (M627 and Tx7000) were planted (25 seeds per pot) on potting compost in plastic pots with cages (6 in diameter and 5.5 in depth). The sorghum M627 line is a strong greenbug resistance line (http://www.dowagro.com/mycogen/sorghum/grain.htm). On the other hand, the sorghum Tx7000 line has a high susceptibility to greenbug phloem-feeding (http://esa.confex.com/esa/2001/techprogram/paper_1814.htm). Seedlings were grown in a greenhouse for 10 days at 29°C and 60% relative humidity in a 14 h-light/10 h-dark photoperiod. Biotype I greenbugs are known to be the most widely spread currently in the USA (Tuinstra et al. 2001), and were raised on susceptible young barley seedlings in a growth chamber for 11 days at 30°C and 60% relative humidity in a 14 h-light/10 h-dark photoperiod.

Aphid infestation on plants

For infestation, greenbugs were placed on sorghum seedlings (10-day-old) with a paint brush. To maintain a heavy infestation, approximately 30 greenbugs were confined on each seedling. Greenbugs were removed at 12, 24, and 72 h after greenbug introduction. Tissues of sorghum seedlings above soil were collected, and then frozen immediately in liquid nitrogen and stored at −80°C prior to use.

Construction of subtractive cDNA libraries

Total RNA was extracted from 72 h greenbug-infested sorghum seedlings of M627, Tx7000, and non-infested M627, respectively, which were collected at the same time. Seedlings were ground into a fine powder in liquid nitrogen and total RNA was extracted using Trizol reagent (Invitrogen, Carlsbad, CA, USA). Then, mRNA was isolated using Poly (A) Purist kit (Ambion, Austin, TX, USA). The cDNA subtraction was carried out using the PCR-Select cDNA subtraction kit (Clonetech, Palo Alto, CA, USA) according to the manufacturer's recommendations. In brief, the two different cDNA subtractions were carried out based on a scheme that mRNA isolated from the greenbug-infested M627, which was used to produce 'tester' cDNA, and mRNA from the infested Tx7000 or non-infested M627, which was used to synthesize 'driver' cDNA, respectively. Two rounds of sequential PCR amplifications were followed on the basis of normalized cDNAs for selective amplification. The resultant PCR products were cloned into the pCR2.1 TA vector (Invitrogen) and transformed into *E. coli* TOP10 cells (Invitrogen). Transformed cells were cultured in a liquid LB medium (Tryptone 10 g, yeast extract 5 g, NaCl 10 g in 1 l LB supplemented with 270 μM ampicillin), and further screening of transformed cells was accomplished by blue-white screening. Transformed cells were stored in a liquid LB medium containing 8% glycerol.

Amplification of cDNA inserts and preparation of cDNA microarray

Differentially expressed cDNAs ligated to the vector pCR2.1 were isolated by PCR amplifications using Nested-1 and -2R primers (Invitrogen). In addition, plasmids from the Arabidopsis functional genomic consortium (AFGC) microarray control set were isolated by PCR amplification, and then purified for use as normalization controls (spikes 1 and 3). Lysates of transformed cells were used directly as DNA templates for PCR amplifications. PCR products were inspected by agarose gel electrophoresis (data not shown). Fifty microliters of each PCR product was mixed with 125 μl ethanol and 5 μl of 5 M NH_4OAc. This mixture was blended by pippeting, and then stored at −80°C for 1 h DNA pellets were recovered by centrifugation at 9,000 rpm for 10 min. After washing with 70% ethanol, the pellets were resuspended in 12 μl distilled water. A concentration of 20× SSC (3 M NaCl, 0.3 M sodium citrate) was added to the resuspended PCR products to a final concentration of 3× SSC. Each cDNA clone was printed three times on amino-silane coated slides (Corning Incorporated, Acton, MA, USA) at the same interval using the GeneMachines OmniGrid 100 system (Genomic solution, Ann Arbor, MI, USA) for technical replication. After printing, the slide was rehydrated with hot vapor and snap dried on a hot plate at 80°C. Then, the slide was baked at 80°C overnight to immobilize the cDNAs.

Preparation of probes and microarray hybridization

Microarray probes were produced from total RNA of seedlings from 72 h-greenbug-infested M627 and Tx7000, as well as from non-infested M627. One hundred micrograms of the total RNA from each sample was converted to cDNA using the Array 350 hybridization kit (Genisphere, Hatfield, PA, USA). In addition, two in vitro transcribed normalization controls (spikes 1 and 3) were prepared using the Riboprobe invitro transcription systems (Promega, Madison, WI, USA), and 100 pg of each control was mixed to the total RNA of each sample for normalization. During reverse transcription, a capture sequence was introduced to the cDNA probes to arrest Cy5 and Cy3 dyes using primers containing a capture sequence. The cDNA probes were mixed together with hybridization buffer (50% formamide, 8× SSC, 1% SDS, 4% Denhardt's solution), LNA dT blocker, and nuclease free water. This mixture was transferred to the slide. A 24×60 mm cover slip (Grace Bio Lab, Bend, OR, USA) was carefully placed on the slide without creating any bubbles, and the slide

was incubated at 42°C overnight. After the hybridization, stringent washes were followed according to the manufacturer's instructions. Each hybridization reaction was repeated twice for biological replication. Probes for the replicate hybridizations were prepared from two independently cultured samples.

Microarray scanning and data analysis

Microarray slides were scanned using the ScanArray Express (Perkin-Elmer, Wellesley, MA, USA) installed with two lasers (Cy5 and Cy3) aiding with the ScanArray Express program (Perkin-Elmer). Normalization of signal intensity values was performed using the internal controls (spikes 1 and 3) spotted on the slide by modulating laser power and photo multiplier tube (PMT) until the intensity ratios of both controls were as close to 1.0 as possible in order to calibrate biased signal intensities of two channels in the beginning of the scan. Further normalization was accomplished by subtracting background noises from each signal intensity of cDNA features printed on the slide using a normalization feature of the GenePix Pro program (version 4.0) (Axon Instrument, Union City, CA, USA). Pre-processing of the normalized microarray data was accomplished using GenePix Auto processor (GPAP) (http://darwin.biochem.okstate.edu/gpap) (Ayoubi et al, unpublished data). This pre-processing included: (1) removal of bad quality spots; (2) removal of data where the fluorescence signal intensities in both channels were less than the background, plus two standard deviations; (3) removal of data where the signal intensities in both channels were less than 200 Relative Fluorescence Units; (4) $\log_2$ transformation of the background subtracted and normalized signal intensity median ratios.

DNA sequencing and database search

The cDNA clones showing differential expression were subjected to sequencing reactions using the BigDye terminator sequencing kit (Applied BioSystem, Foster City, CA, USA) and ABI Model 3700 DNA Analyzer (Applied BioSystem). The database search was performed on the basis of the cDNA sequences using BLASTX and BLASTN. BLASTN was used in the case of absence of any matched hits when performing BLASTX. All cDNA sequences were submitted to the GenBank dbEST, and accession numbers are listed in Table 1.

Northern-blot analysis

Total RNA was isolated from seedlings collected after three different time points of greenbug infestation (12, 24, and 72 h), as well as from non-treated control sorghum materials (10-day-old) in the same manner as above, respectively. Approximately 10 μg of total RNA per sample was fractionated in a 1% agarose gel containing 1.1 M formaldehyde, and then transferred to an Hybond-N^+ membrane (Amersham Biosciences, Piscataway, NJ, USA) using the alkaline solution (3 M NaCl and 0.01 N NaOH) transfer method. Probes were labeled with ^{32}P-dCTP (Perkin-Elmer) using PCR amplification of cDNA inserts from the pCR2.1 vector and hybridized to the membrane soaked with 2 ml of the UltraHyb buffer (Ambion) at 42°C overnight. Then, the hybridized blots were washed with 2× SSC/0.1% SDS at 65°C and 0.1× SSC/0.1% SDS at 60°C and exposed on a Kodak BioMax MS film (Kodak, Rochester, NY, USA) at −80°C overnight.

Results

Expression profiling of sorghum genes responsive to greenbug phloem-feeding

In this study, two different sorghum lines known to possess different characteristics on greenbug resistance were used to profile greenbug responsive genes for better understanding of sorghum defense mechanisms against greenbug feeding. Seedlings of the sorghum M627 line showed a few necrotic spots and maintained healthy green color after 72 h of greenbug-infestation, but those of the Tx7000 line exhibited widespread necrotic spots and severe wilting under the same treatment (Fig. 1b, c). Two subtractive cDNA libraries enriched in genes responsive to greenbug feeding were constructed from the sorghum lines, M627 and Tx7000. A collection of 3,508 cDNA clones were obtained from the cDNA libraries and printed on specially designed glass slides for the microarray analyses.

Based on the collected cDNAs, two microarray analyses were performed. Each microarray analysis was designed to investigate the expression patterns of transcriptome profiles from two different combinations of sorghum plants, greenbug infested M627 (Mi) versus non-greenbug infested M627 (Mni) and Mi versus greenbug infested Tx7000 (Ti). In the microarray analyses, expression profiles of sorghum genes showing induction or suppression in response to greenbug feeding were investigated. To increase the reliability and consistency of the microarray analyses, application of multiple replicates was adopted following the suggestion (Ting Lee et al. 2000). To perform each microarray analysis, two independently prepared biological replicates and three technical replicates were used to minimize the versatility of results. To avoid technical bias of intensity ratios between Cy5- and Cy3-fluors, the intensity ratio of each clone was normalized using two normalization control features (spikes 1 and 3) synthesized from two human genes encoding B-cell receptor-associated protein and myosin light chain 2, respectively, and spotted on the slide. In addition, a significance of correlation in the expression fold changes among the replicates of each cDNA was considered by the statistical analyses

Table 1 Measurement of changes in the expression of genes responsive to greenbug phloem-feeding

Clone	Putative function/homology/species[a]	Signal intensity ratios[b,c]		Score/e-value	Accession No.[d]
		M627i vs M627ni	*M627i vs Tx7000i*		
	Abiotic stress				
MM1	Soluble starch synthase_*Sorghum bicolor*	10.476	-16.089	120/2e-26	DR831413
MT158	Phytochelatin synthetase-like protein 1_*Sorghum bicolor*	1.238	1.796	54/2e-04	DR831443
MT32	ASR2 protein _*Oryza sativa*	2.155	-4.228	68/1e-10	DR831414
MM15	Heat shock protein70_*Oryza sativa*	3.964	-2.255	213/2e-54	DR831415
	Cell wall fortification				
MT40	Delta1 pyrroline-5-carboxylate dehydrogenase_*Oryza sativa*	1.936	-3.88	102/4e-21	DR831418
MT29	Glycosyl transferase_*Sorghum bicolor*	2.01	-3.595	52.8/3e-06	DR831419
MM108	Glycosyl transferase-like protein_*Oryza sativa*	2.043	-1.183	115/3e-25	DR831416
MT112	2-dehydro-3deoxyphosphooctonate aldolase_*Oryza sativa*	-1.319	2.439	86.3/3e-16	DR831421
MM25	Caffeic acid O-methyltransferase_*Sorghum bicolor*	3.568	2.882	64.7/8e-10	DR831420
MT89	d-TDP glucose dehydratase_*Phragmites australis*	1.022	3.069	271/1e-71	DR831422
MT80	Cellulose synthase catalytic subunit10_*Zea mays*	1.669	3.297	61.9/7e-07	DR831429
MT69	Cellulose synthase-7_*Zea mays*	-1.534	3.635	87.7/7e-15	DR831430
MM36	Proline rich protein_*Zea mays*	4.649	4.983	67/3e-10	DR831431
	Cell maintenance				
MM75	Adenine nucleotide translocator_*Zea mays*	2.637	-2.041	122/3e-27	DR831565
MT33	Aspartate aminotransferase_*Oryza sativa*	1.141	-4.09	175/4e-43	DR831432
MT179	Adenine phosphoribosyltransferase-form2_*Oryza sativa*	1.05	-2.301	178/1e-43	DR831433
MT50	RING-H2 finger protein RHG1a_*Oryza sativa*	1.003	-2.286	135/4e-31	DR831434
MM113	Actin_*Triticum aestivum*	1.796	-1.643	200/1e-50	DR831435
MM67	ATP/ADP translocase_*Zea mays*	2.572	-1.23	94.4/9e-19	DR831436
MM58	Ubiquitin ligase SINAT5_*Oryza sativa*	2.856	-1.121	113/2e-24	DR831437
MM104	ADP-glucose pyrophosphorylase small subunit_*Zea mays*	1.97	-1.056	127/1e-28	DR831566
MM93	60S ribosomal protein_*Oryza sativa*	2.151	1.07	207/1e-52	DR831438
MM110	Ribosomal protein S7_*Oryza sativa*	1.838	1.189	140/2e-32	DR831439
MM9	40S ribosomal protein_*Oryza sativa*	4.887	1.218	173/2e-42	DR831440
MM106	60S ribosomal protein L24_*Oryza sativa*	1.989	1.252	119/3e-26	DR831441
MM30	CTP synthase_*Oryza sativa*	-2.264	1.645	140/1e-32	DR831442

provided in the GPAP. In this study, genes were considered to be differentially regulated if the intensity ratios of cDNA clones from the microarray analyses showed more than 1.8-fold changes toward up or down. Two scatter plots representing signal intensity patterns of features on the slide for the microarray analyses are shown (Fig. 2a, b). On an average, approximately 18% (651/3,508) of the transcripts were found to be up- or down regulated more than 1.8-fold by greenbug feeding in the microarray analyses. In total, we obtained 157 genes that showed greater than a 1.8-fold induction or suppression after removal of redundant transcripts and statistically non-significant data. It is believed that these genes are involved directly or indirectly in sorghum defense responses against greenbug attack.

Co-regulation patterns of greenbug responsive genes

In the two different microarray analyses, some genes responsive to greenbug feeding were found to be co-regulated in both microarray analyses. The microarray analyses showed 72 upregulated genes in comparison of Mi to Mni, and 82 upregulated genes in Mi–Ti comparison. Among the upregulated genes, 11 genes were commonly up regulated in both microarray analyses (Fig. 3a). The 11 genes commonly upregulated belong to various functional categories such as cell wall fortification, defense, signal transduction, oxidative burst/stress, development, cell maintenance, and unknown function. On the other hand, 12 genes were suppressed in the microarray analysis between Mi and Mni, and 42 genes were down regulated in the microarray analysis between Mi and Ti in response to greenbug feeding. Out of a total of 54 down regulated genes, two genes encoding catalase and WD domain G-beta repeat containing protein were commonly down regulated in both microarray analyses (Fig. 3b).

Functional classification of genes

A total of 157 genes differentially regulated in response to the greenbug feeding were listed and categorized according to the putative function of each gene

Table 1 (Contd.)

MT170	RNA polymerase subunit_*Oryza sativa*	-1.179	1.834	149/3e-35	DR831444
MT147	ATP-dependent transmembrane transporter_*Oryza sativa*	1.385	2.142	137/8e-32	DR831445
MM54	Alpha tubulin_*Zea mays*	2.59	2.732	150/1e-35	DR831446
MT101	Small nuclear ribonucleoprotein polypeptideE_*Oryza sativa*	-1.115	2.811	131/6e-30	DR831447
MT95	Suppressor of actin1_*Oryza sativa*	-1.134	2.904	38.1/0.083	DR831448
MT96	Bundle sheath cell specific protein1_*Zea mays*	-1.613	3.158	106/5e-22	DR831449
MT174	NOD26-like membrane integral protein_*Zea mays*	1.29	5.159	158/1e-37	DR831450
MM20	Histone H2A_*Zea mays*	-2.4	5.521	74.7/8e-13	DR831451
MT146	Peroxisomal membrane protein_*Oryza sativa*	1.223	2.015	225/6e-58	DR831452
MT42	Ribosomal protein L2_*Eucalyptus globules*	-1.173	4.019	56/4e-05	DR831517
MM4	Inorganic phosphate transporter_*Agaricus bisporus*	-9.573	N/A	52/5e-04	DR831453
MM22	Beta tubulin_*Zea mays*	-2.84	N/A	52/7e-04	DR831454
	Defense-related				
MT4	Beta glucosidase_*Oryza sativa*	3.899	-22.1	150/2e-35	DR831570
MM2	Sulfur-rich/thionin-like protein_*Triticum aestivum*	13.251	-5.692	79.7/2e-14	DR831455
MT20	Glucan endo-1,3-beta-glucanase_*Zea mays*	2.218	-4.35	52/5e-06	DR831456
MT31	S-like RNase_*Oryza sativa*	1.801	-3.74	38.1/0.083	DR831457
MM37	Cysteine proteinase inhibitor_*Sorghum bicolor*	3.324	-2.823	93.6/2e-18	DR831459
MM76	Cysteine proteinase_*Zea mays*	2.652	-2.539	99.6/4e-18	DR831458
MT44	Polyphenol oxidase_*Triticum aestivum*	3.573	-2.228	199/4e-50	DR831460
MT177	Wilms' tumor-related protein QM_*Oryza sativa*	1.342	-2.006	120/1e-26	DR831461
MM103	Legumain-like protease_*Zea mays*	2.105	-1.945	223/4e-57	DR831462
MM79	Endo-1,4-beta glucanase Cel1_*Hordeum vulgare*	2.621	-1.829	224/2e-57	DR831463
MM78	Wound inductive gene_*Oryza sativa*	2.621	-1.763	120/2e-26	DR831464
MM86	Multiple stress responsive zinc-finger protein_*Oryza sativa*	2.428	-1.659	169/3e-41	DR831465
MM95	Oxysterol-binding protein_*Arabidopsis thaliana*	2.135	-1.647	271/4e-72	DR831466
MM71	Cytochrome P450-like protein_*Sorghum bicolor*	2.757	-1.268	365/6e-100	DR831467
MM31	Cytochrome P450 monooxygenase_ *Zea mays*	-2.253	1.803	117/1e-25	DR831468
MM73	Xa1-like protein_*Sorghum bicolor*	2.39	1.866	211/8e-54	DR831470
MT162	OTU-like cystein domain containing protein_*Oryza sativa*	-1.066	1.867	69.7/2e-11	DR831471
MT35	Cytochrome P450 monooxygenase_*Oryza sativa*	1.008	3.655	213/1e-54	DR831469
	Development				
MM65	24kDa seed maturation protein_*Oryza sativa*	3.18	-1.955	210/1e-53	DR831472
MT121	Auxin induced protein_*Saccharum*-hybrid cultivar	1.8	2.5	84.3/1e-15	DR831473
MT103	GA-induced cysteine-rich protein_*Petunia x hybrida*	-1.882	2.856	67.8/9e-11	DR831474
MT88	GH1 protein or auxin regulated protein_*Oryza sativa*	1.471	2.924	52.0/6e-04	DR831475
	Oxidative burst/stress				
MM13	Peroxidase_*Zea mays*	9.474	-11.464	244/1e-63	DR831476
MM46	Catalase_*Oryza sativa*	-2.84	-8.427	124/8e-28	DR831477
MM51	Glutathione S-transferase_*Ixodes ricinus*	3.541	-3.242	52/7e-04	DR831478
MT178	Quinone oxidoreductase_*Oryza sativa*	2.242	-2.117	334/1e-90	DR831479
MT90	Catalase isozyme3_*Zea mays*	3.605	-3.017	119/3e-26	DR831480
	Photosynthesis-related				
MM23	NADP-specific isocitrate dehydrogenase_*Oryza sativa*	3.315	-3.053	233/2e-60	DR831481
MT38	Citrate synthase, glyoxisomal precursor_*Oryza sativa*	1.429	-2.87	223/2e-57	DR831482
MM60	Enolase_*Zea mays*	3.114	-2.285	187/7e-47	DR831483
MM89	Chloroplast thylakoidal processing peptidase_*Oryza sativa*	2.387	-1.486	92.8/3e-18	DR831484
MM96	RuBisco subunit binding protein beta subunit_*Zea mays*	2.231	1.005	173/1e-42	DR831485
MM56	Lipoic acid synthase_*Arabidopsis thaliana*	4	1.105	74.3/1e-12	DR831486

Table 1 (Contd.)

MM33	Type II chlorophyll a/b binding protein_*Sorghum bicolor*	-1.808	1.347	129/2e-27	DR831487
MM97	Sedoheptulose-1,7-bisphosphatase precursor_*Oryza sativa*	2.199	1.56	212/3e-54	DR831488
MM5	Cytochrome b6/f complex subunit5_*Oryza sativa*	-3.006	1.97	44.7/8e-04	DR831489
MT176	Mannose 6-phosphate reductase_*Oryza sativa*	1.339	1.992	389/6e-107	DR831490
MT152	Photosystem1 reaction center subunit2_*Oryza sativa*	-1.069	2.027	249/4e-65	DR831491
MT155	Plastid ribosomal protein L19 precursor_*Oryza sativa*	1.764	2.034	216/5e-55	DR831492
MT151	Photosystem I chain D precursor_*Hordeum vulgare*	1.257	2.123	97.1/2e-19	DR831493
MT125	Ribosomal protein chloroplast-like_*Oryza sativa*	1.416	2.334	114/2e-24	DR831494
MT79	Photosystem2 10k protein_*Oryza sativa*	-1.08	2.558	131/1e-27	DR831568
MT23	Ferredoxin_*Zea mays*	-1.866	3.145	160/3e-38	DR831495
MM11	Chlorophyll a/b binding protein precursor_*Oryza sativa*	-2.382	3.154	224/2e-57	DR831496
MT68	29kDa ribonucleoprotein A chloroplast precursor_*Oryza sativa*	1.293	3.771	242/6e-63	DR831569
MT54	SecA-type chloroplast protein transport factor_*Oryza sativa*	1.275	3.97	164/1e-39	DR831427
MT28	Harpin inducd protein_*Oryza sativa*	1.131	3.896	240/3e-62	DR831515
	Signal transduction				
MT18	Ras-GTPase activating protein binding protein2_*Oryza sativa*	11.959	-14.113	99.4/3e-20	DR831498
MT5	CCR4-NOT transcription complex subunit7_*Oryza sativa*	3.254	-7.989	43.1/0.003	DR831499
MM24	Gamma2 subunit of voltage gated Ca2+ channel_*Mus musculus*	5.657	-3.458	199/2e-50	DR831417
MM100	ADP-ribosylation factor_*Oryza sativa*	2.155	-3.053	270/3e-71	DR831500
MM19	WD domain, G-beta repeat containing protein_*Oryza sativa*	-2.172	-2.803	41/0.007	DR831423
MM41	Phospholipase_*Oryza sativa*	4.26	-2.481	252/3e-66	DR831424
MT43	Aci-reductone dioxygenase-like protein_*Oryza sativa*	1.853	-2.42	166/2e-40	DR831425
MM62	Stearoyl-acyl-carrier protein desaturase_*Oryza sativa*	2.834	-1.9	97.8/9e-20	DR831426
MM77	Steroid membrane binding protein_*Oryza sativa*	2.743	-1.472	170/1e-41	DR831428
MM107	ADP-ribosylation factor1-like_*Arabidopsis thaliana*	2.029	-1.389	198/3e-50	DR831501
MM81	Glycine-rich RNA-binding protein_*Sorghum bicolor*	2.579	-1.26	184/4e-44	DR831502
MM85	Methionine adenosyltransferase_*Hordeum vulgare*	2.444	1.302	125/6e-28	DR831503
MM82	Omega-3 fatty acid desaturase_*Zea mays*	2.585	1.348	493/e-138	DR831504
MT171	Phosphatidic acid phosphatase beta-like_*Oryza sativa*	1.066	1.794	146/4e-34	DR831505
MT166	Phosphoinositide kinase_*Oryza sativa*	1.029	1.83	310/2e-83	DR831506
MT153	GTP-binding protein typA_*Oryza sativa*	N/A	1.979	213/5e-54	DR831507
MM83	Wheat adenosylhomocysteinase-like protein_*Oryza sativa*	2.518	2.003	125/3e-28	DR831508
MT159	ARF GTPase-activating domain containing protein_*Oryza sativa*	N/A	2.007	190/2e-47	DR831509
MT143	GTP-binding protein RIC2_*Oryza sativa*	1.157	2.218	308/5e-83	DR831510
MT123	Ankyrin like protein_*Oryza sativa*	1.058	2.874	304/2e-81	DR831511
MT59	Acid cluster protein 33_*Oryza sativa*	N/A	3.202	191/6e-48	DR831512
MT65	Inorganic pyrophosphatase_*Oryza sativa*	1.207	3.461	179/6e-44	DR831513
MT63	GDSL-motif lipase/hydrolase-like protein_*Oryza sativa*	1.016	3.523	41.6/0.008	DR831514
MT37	Acyl-CoA binding protein_*Oryza sativa*	-1.241	3.939	154/2e-36	DR831516
MT26	Gamma-2 subunit of voltage-gated Ca2+ channel_*Mus musculus*	-1.493	4.147	77.4/1e-13	DR831518
MT13	Phytosulfokine receptor precursor_*Oryza sativa*	-1.607	4.807	152/4e-36	DR831519
	Unknown function				
MM7	OSJNBb0022F23.4_*Oryza sativa*	8.363	-6.945	83.2/2e-15	DR831520
MT19	Unknown_*Glycine max*	1.247	-6.238	140/3e-32	DR831521
MT21	Hypothetical protein_*Candida albicans*	2.071	-4.847	49/8e-05	DR831522
MT7	No similarity found	N/A	-4.731		DR831523
MT8	OSJNBa0016O02.6_*Oryza sativa*	N/A	-4.469	119/9e-26	DR831524
MM35	No similarity found	4.823	-1.937		DR831525
MM16	OSJNBb0014D23.16_*Oryza sativa*	5.926	-1.706	103/5e-21	DR831526

Table 1 (Contd.)

MM14	No similarity found	4.095	-1.617		DR831527
MM44	No similarity found	3.585	-1.555		DR831528
MM27	No similarity found	4.368	-1.432		DR831529
MM109	At3g26710_*Arabidopsis thaliana*	1.858	1.293	116/2e-25	DR831530
MM18	No similarity found	-3.326	1.302		DR831531
MM68	Hypothetical protein_*Sorghum bicolor*	2.892	1.715	61.2/9e-09	DR831532
MT168	No similarity found	-1.611	1.829		DR831533
MM17	No similarity found	4.955	1.852		DR831534
MT156	Unnamed protein product_*Triticum aestivum*	1.358	1.861	290/2e-77	DR831535
MT161	Ab2-057_*Rattus norvegicus*	1.123	1.865	113/2e-24	DR831536
MT160	No similarity found	-1.134	1.888		DR831537
MT164	No similarity found	-1.14	1.913		DR831538
MT154	No similarity found	1.007	1.925		DR831539
MM99	OSJNBa0093F16.13_*Oryza sativa*	2.263	1.932	148/4e-35	DR831540
MT139	OSJNBa0017P10.11_*Oryza sativa*	1.184	1.935	97.8/9e-20	DR831541
MT149	No similarity found	1.155	2.079		DR831542
MT104	No similarity found	1.07	2.081		DR831543
MT144	No similarity found	-1.487	2.176		DR831544
MT141	Expressed protein_*Oryza sativa*	-1.364	2.216	44.7/8e-04	DR831545
MT113	No similarity found	1.358	2.305		DR831546
MT92	Unnamed protein product_*Hordeum vulgare*	1.297	2.415	189/3e-47	DR831547
MT97	No similarity found	N/A	2.486		DR831548
MT93	Unknown protein_*Oryza sativa*	-1.273	2.5	73/3e-12	DR831549
MT131	No similarity found	-1.005	2.512		DR831550
MT127	No similarity found	-1.001	2.565		DR831551
MT106	Unknown protein_*Oryza sativa*	1.194	2.726	57/2e-07	DR831552
MT105	OSJNBb0006N15.13_*Oryza sativa*	-1.285	2.834	69.3/3e-11	DR831553
MM87	Unknown protein_*Oryza sativa*	2.412	2.904	62/6e-09	DR831554
MT85	Unknown_*Saccharomyces cerevisiae*	1.014	3.145	76.6/2e-13	DR831555
MT77	Unknown protein_*Oryza sativa*	2.349	3.317	126/2e-28	DR831557
MT75	OSJNBa0081L15.5_*Oryza sativa*	-1.178	3.504	42.4/0.004	DR831556
MT72	No similarity found	1.12	4.211		DR831558
MT24	No similarity found	-1.186	4.243		DR831559
MT16	Unknown protein_*Oryza sativa*	1.845	4.608	90.9/2e-17	DR831560
MT14	No similarity found	1.259	4.611		DR831561
MT12	OSJNBa0033G05.15 *Oryza sativa*	1.264	5.053	147/2e-34	DR831562
MT173	Unnamed protein product_*Kluyveromyces lactis*	-1.266	5.367	163/2e-39	DR831563
MT3	Unknown protein_*Oryza sativa*	1.199	7.15	103/5e-21	DR831564
MT175	No similarity found	1.024	2.823		DR831567

[a]BLASTX was used to determine homologous genes and putative functions of genes. BLASTN was used in case of failure to return any hits by BLASTX

[b]Values of signal intensity ratios showing up- or down regulation more than a 1.8-fold were shaded with pale blue or yellow as in order. The values of the signal intensity ratio were determined by calculating a median value of signal intensity ratios of replicates

[c]*N/A* indicates 'Not available' due to the low significance of data

[d]GenBank accession number. All cDNA sequences were submitted to the GenBank database

(Table 1). The signal intensity ratios of these genes from the two microarray analyses were also provided in Table 1. The putative functions of genes were inferred from metabolic processes known to be related to each gene. Even though some genes were involved in multiple metabolic processes, they were classified according to their main roles in plant metabolism. The sorghum genes responsive to greenbug feeding were classified into nine functional categories such as direct defense, signal transduction, cell wall fortification, oxidative burst/stress, photosynthesis, development, cell maintenance, abiotic stress, and unknown function. The genes with

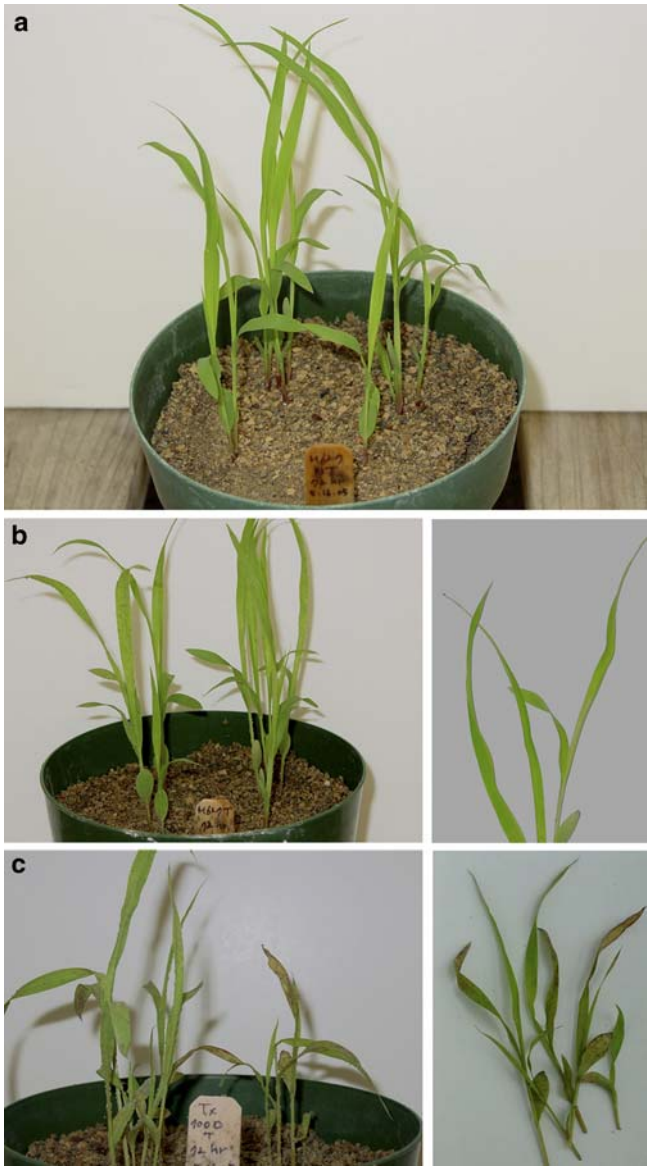

Fig. 1 Phenotypes of seedlings from different sorghum lines after 72 h greenbug infestation. **a** Seedlings of sorghum line M627 with no greenbug infestation, harvested at the same time point with (**b**) and (**c**). **b** Phenotype of M627 seedlings after 72 h greenbug infestation (*left*). Closer view of 72 h greenbug infested M627 seedlings (*right*). **c** Phenotype of Tx7000 seedlings after 72 h greenbug infestation (*left*). Closer view of 72 h greenbug infested Tx7000 seedlings (*right*)

unknown function occupy the greatest category, and the group of signal transduction is ranked as the second largest group, followed by the cell maintenance (Fig. 4).

Defense-related genes

A group of genes involved in biosynthesis of defense molecules was either up- or down regulated by greenbug feeding (Table 1). In total, 18 genes were involved in direct defense responses and were differentially expressed in both microarray experiments. These genes encode well-known defense molecules, including cysteine proteinase inhibitors (CPIs), polyphenol oxidase, legumain, glucosidase, thionin, glucanase, cysteine proteinase and S-like RNase. A gene encoding CPI, a well-known plant defense molecule against insect herbivory (Botella et al. 1996), was induced from the earlier stage (12 h) of greenbug infestation (Fig. 5) and maintained at a high level of induction until 72 h post-infestation. Polyphenol oxidase (PPO) catalyzes biosynthesis of active quinones which are toxic to herbivores and pathogens due to their ability to produce indigestible modified amino acids and proteins (Li and Steffens 2002). The *PPO* gene was induced from 72 h of greenbug infestation (Fig. 5). Thionin is a cysteine-rich antimicrobial protein induced by infection of fungi and bacteria (Oh et al. 1999). Intense induction of the thionin gene (*Thi*) was observed from 12 h to 72 h of greenbug infestation (Fig. 5). The genes encoding Xa1 protein (*Xa1*) and cytochrome P450 protein (*CYP*) were co-upregulated in both microarray analyses. Xa1 is a bacterial blight-resistance protein and known to confer resistance against pathogen attack by recognizing pathogen-related particles and eliciting defense responses in the cytosol (Yoshimura et al. 1998). The expression of the *Xa1* gene was induced from 72 h of greenbug infestation, reversed from suppression at 12 and 24 h (Fig. 5). The gene encoding cysteine proteinase (*CP*) was induced from 72 h of greenbug infestation (Fig. 5). Pechan et al. (2000) demonstrated that the *CP* gene was induced by larval feeding, and CP participated in inhibition of lepidopteran larvae growth in maize.

Genes involved in cell wall fortification

Nine genes involved in cell wall fortification were up- or down regulated by greenbug infestation (Table 1). The genes encoding caffeic acid *O*-methyltransferase (*COMT*) and proline-rich protein (*PRP*) were co-upregulated in both microarray analyses. COMT participates in lignification of cell walls (Morreel et al. 2004), and PRP is known to be one of the structural components of cell walls, and involved in cell wall reinforcement (Vignols et al. 1999). The *COMT* gene was induced after 72 h of greenbug infestation in both microarray analyses (Figs. 5, 6), and the *PRP* gene was upregulated from 12 h after greenbug infestation (Fig. 6).

Signal transduction

As a whole, 26 genes involved in signal transduction were expressed differentially in response to greenbug feeding (Table 1). The number of genes in this category makes up for the second greatest category, next to the category of unknown function. Among these genes, a gene-encoding Ras-GTPase activating protein binding protein (*Ras*) was significantly up- or down

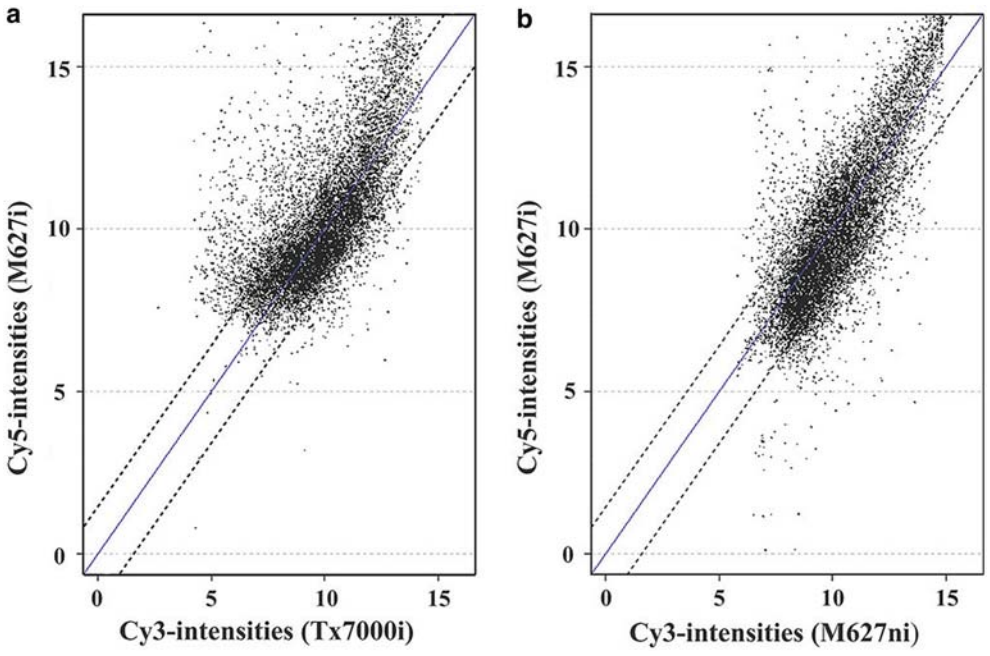

Fig. 2 Two scatter plots showing distribution of normalized expression patterns of cDNA clones following the microarray hybridizations. **a** Scatter plot of normalized log 2 intensities of Cy3 (Tx7000 greenbug-infested) versus log 2 intensities of Cy5 (M627 greenbug-infested). **b** Scatter plot of normalized log 2 intensities of Cy3 (M627 non-greenbug infested) versus log 2 intensities of Cy5 (M627 greenbug-infested). *Solid line* represents a 1:1 ratio of signal intensity. *Dotted lines* indicate 1.8-fold induction (*upper-dot line*) or suppression (*lower-dot line*) of gene expression. Normalized intensity ratios are shown for all features prior to data filtering (intensity ratios of replicates were included)

regulated. The Ras-GTPase is known to play a crucial role in controlling mitogen-activated protein kinases (MAPKs) and transduces diverse signals in animals (Shields et al. 2000). In Arabidopsis, Ras-GTPase is absent and the role of Ras-GTPase is carried out by Rop-GTPase (Li et al. 2001). The expression of *Ras* showed reverse patterns between the two microarray experiments. In the microarray analysis between Mi and Mni, the *Ras* gene was induced from 72 h of greenbug infestation, but suppressed in the analysis between Mi and Ti from 12 h of greenbug infestation. This suppression of *Ras* resulted from higher upregulation of *Ras* in Ti than that of Mi at 72 h of the infestation (Figs. 5, 6). A gene-encoding ankyrin-induced protein was upregulated. Ankyrin regulates the SA-dependent defense reactions, including systemic acquired resistance (Lu et al. 2003).

Oxidative burst/stress

The genes encoding peroxidase (*PX*), gluthathion-*S*-transferase (*GST*), catalase (*CAT*), and quinone oxidoreductase (*QR*) were up- or down regulated by greenbug feeding (Table 1). Both PX and CAT play a key role in controlling ROS concentration, leading to oxidative signal transductions (Kawano 2003). The *CAT* gene was suppressed from 12 h of greenbug infestation, but the *PX* gene was induced from 12 h of greenbug infestation

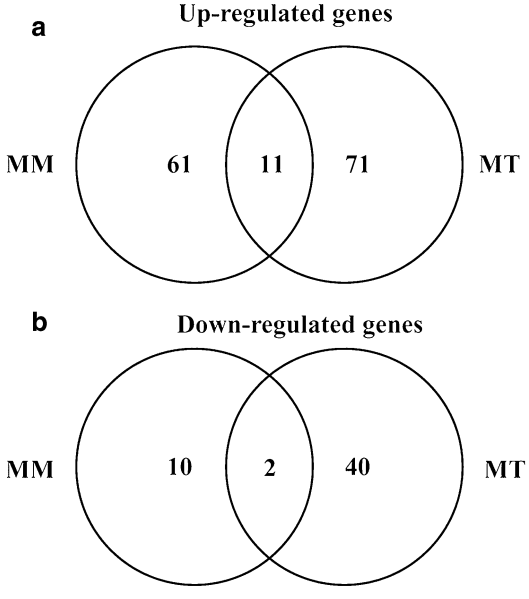

Fig. 3 Venn diagrams of genes differentially expressed by greenbug feeding in the two different microarray analyses. *MM* indicates the microarray analysis between greenbug infested M627 and non-greenbug infested M627, and *MT* indicates the microarray analysis between greenbug infested M627 and greenbug infested Tx7000. **a** Numbers of genes which were induced more than 1.8-fold in MM and MT. **b** Numbers of genes which were suppressed more than 1.8-fold in MM and MT

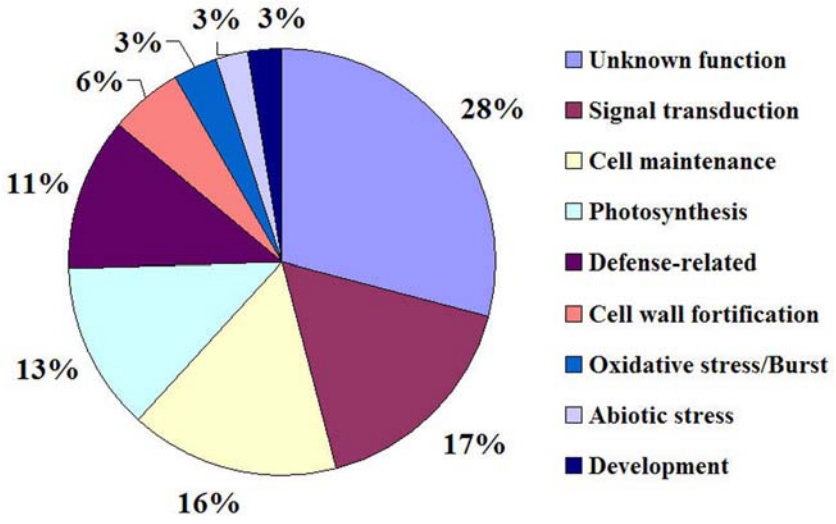

Fig. 4 Functional categories of the sorghum genes responsive to greenbug phloem-feeding. In pie chart, *values of percentage* indicate the proportion of a number of genes in each category to total number of genes (157 genes), and the functional categories were annotated (*right*)

and reached a peak point at the 24 h time point (Fig. 5). QR scavenges toxic free radical semiquinones using divalent reduction, and was induced by oxidative stress in Arabidopsis (Mano et al. 2002).

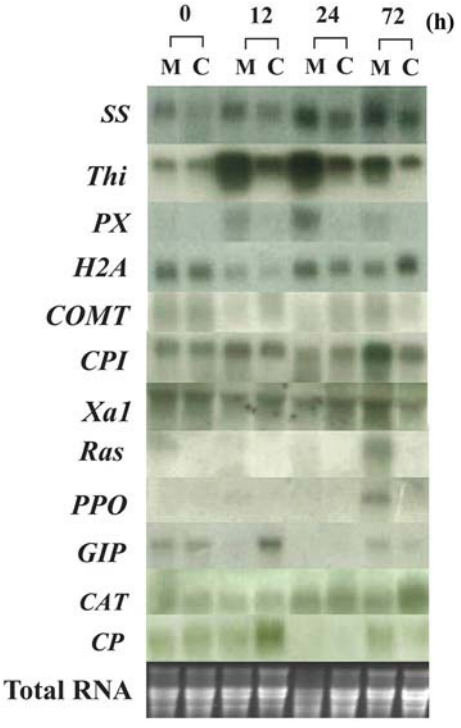

Fig. 5 Northern-blot confirmation of the cDNA microarray analysis. Total RNAs were extracted from greenbug-infested M627 and -uninfested M627 sorghum seedlings at 0, 12, 24, and 72 h after greenbug infestation for northern-blot analysis. Equilibrium of RNA loading was verified by intensity of total RNA bands. *M* M627 greenbug infested; *C* M627 untreated controls; *SS* starch synthase; *Thi* sulfur rich/thionin protein; *PX* peroxidase; *H2A* histone H2A; *COMT* caffeic-acid *O*-methyltransferase; *CPI* cysteine proteinase inhibitor; *Ras* Ras GTPase activating protein binding protein; *PPO* polyphenol oxidase; *GIP* gibberellin induced protein; *CAT* catalase; *CP* cysteine proteinase

Abiotic stress responsive genes

Four genes encoding starch synthase (*SS*), heat shock protein (*Hsp*), phytochelatin synthetase (*PCS*), and ABA-water stress-ripening-induced protein (*ASR*) showed differential regulation in response to greenbugs. Up regulation of the *SS* gene was reported on wheat under heat stress (Majoul et al. 2004), and rapid changes in expression of the *SS* gene were also reported in water-

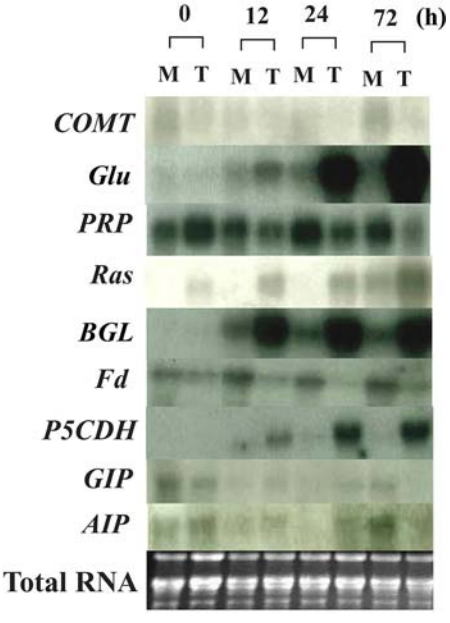

Fig. 6 Northern-blot confirmation of the cDNA microarray analysis. Total RNAs were extracted from greenbug infested-M627 and -Tx7000 sorghum seedlings at 0, 12, 24, and 72 h after greenbug infestation for northern-blot analysis. Equilibrium of RNA loading was verified by intensity of total RNA bands. *M* M627 greenbug infested; *T* Tx7000 greenbug infested; *Glu* beta-glucosidase; *PRP* proline rich protein; *BGL* beta-glucanase; *Fd* Ferredoxin; *P5CDH* pyrroline-5-carboxylate dehydrogenase; *AIP* auxin induced protein

stressed wheat plants to control photoassimilation (Ahmadi and Baker 2001). The *SS* gene was induced from the 12 h of greenbug infestation, and gradually increased its induction as extension of the infestation (Fig. 5). Induction of the *ASR* gene for protection of plant DNA under water-stressed condition is known to be controlled by the phytohormone ABA (Riccardi et al. 1998). Two sorghum genes such as aldehyde oxidase gene and drought-, salt-, and low temperature responsive gene (*DRT*), which are known to be regulated by ABA, were profiled in response to greenbugs (Zhu-Salzman et al. 2004). Considering our results and previous reports, it is plausible that ABA participates in regulating sorghum defense responses against greenbugs.

Cell maintenance involved genes

As shown in Table 1, 25 genes involved in cell maintenance showed differential expression by greenbug infestation. Several genes encoding 40S- and 60S-ribosomal protein subunits were upregulated in both microarray analyses. Differential expression of genes encoding alpha- and beta-tubulin was also shown. A gene encoding alpha tublin was upregulated by application of *Cis*-jasmone, a well-known plant hormone involved in defenses against insect herbivory (Birkett et al. 2000). An actin-encoding gene was also upregulated. Compositional changes of actin cytoskeletons in plant cells were involved in defense events during pathogenesis (Kobayashi and Hakuno 2003). A gene encoding aspartate aminotransferase (*AAT*) was down regulated. AAT was known to play a pivotal role in nitrogen and carbon metabolism, especially in C_4-plants and legumes (Silvente et al. 2003), and spression of the *AAT* gene was reported in Penjalinan plants under a drought condition (Aroca et al. 2003). The gene encoding histone H2A (*H2A*) was induced from 12 to 24 h of greenbug infestation, and reversed to suppression from 72 h of greenbug infestation (Fig. 5). Intense induction of *H2A* gene was reported in drought stressed hot pepper plants (Park et al. 2003).

Genes involved in development

A group of genes encoding auxin induced protein (*AIP*), GA induced protein (*GIP*), and seed maturation protein was either up- or down regulated by greenbug feeding. A gene encoding AIP was co-upregulated in both microarray analyses (Table 1). The *GIP* gene was induced from 72 h of greenbug infestation, and the *AIP* gene was also upregulated from 72 h of greenbug infestation (Figs. 5, 6). The plant hormones such as auxin and GA have been widely known to be involved in the plant development. They also negatively affect expression of several defense genes in plants, and show antagonistic relationships with defense-related hormones such as ABA and ET (Mayda et al. 2000).

Photosynthesis-related genes

A number of genes involved in photosynthesis were up- or down regulated by greenbug feeding (Table 1). Ferredoxin (Fd) is an iron–sulfur containing protein mainly located in chloroplast photosystem I, and promotes harpin-mediated HR (Dayakar et al. 2003). The *Fd* gene was induced from 12 h of greenbug infestation (Fig. 6). Various biotic- and abiotic-stresses, including plant hopper phloem-feeding in rice, cause suppression of photosynthesis (Watanabe and Kitagawa 2000). This suppression is attributed to the redistribution of energy to reinforce defense responses (Zhu-Salzman et al. 2004). Our data showed prevalent induction of photosynthesis-related genes in the microarray analysis between Mi and Ti (Table 1). It is plausible that severe damage inflicted on seedlings of Ti by greenbug feeding caused irrecoverable failure of photosynthetic machineries, leading to a decrement of expression of photosynthesis-related genes in Ti.

Genes of unknown function

The genes with unknown function ranked as the largest one in all nine categories (Fig. 4). A total of 46 cDNAs failed to hit any matched sequences from the GenBank databases by the BLAST search or matched to sequences whose functions have not been characterized yet. Five genes of unknown function were co-upregulated, and two were reversely regulated in the two microarray analyses (Table 1). Some of them showed strong up- or down regulation by greenbug feeding. This implies that these genes are intimately involved in regulation of sorghum defense responses against greenbugs.

Discussion

In this study, two sorghum lines possessing contrastive characteristics of greenbug resistance were used for cDNA subtraction and microarray experiments to maximize the possibility of profiling genes responsive to greenbug feeding. In these comparative analyses with a 3.5 K cDNA microarray, a total of 157 transcripts were identified to be responsive to greenbug feeding. The resultant profiles are more comprehensive than other aphid-induced gene profiles reported earlier (Moran et al. 2002; Voelckel et al. 2004; Zhu-Salzman et al. 2004). These comparative approaches not only allowed us to profile genes which were unable to be identified in the previous studies, but also confirm the genes previously identified to be responsive to greenbug feeding. Compared to a previous study (Zhu-Salzman et al. 2004) conducted with a similar purpose, our results showed a high level of consistent results, but also exhibited some novel data contributing to a better understanding of plant defense responses against greenbugs. It is believed that most added results resulted from the use of two

contrastive sorghum lines showing strong greenbug-resistance and -susceptibility. Unlike previous reports from Zhu-Salzman et al. (2004) and other groups, which focused on aphid-induced responses of a susceptible host plant, this study showed differential responses against greenbugs by comparative analyses between resistant and susceptible lines. Thus, the defense responsive genes identified in the resistant source may contribute to a strong resistance to greenbugs when compared with the susceptible line.

Phloem-feeding aphids represent a special model in studies of plant–insect interactions. When aphids attack host plants, they penetrate plant tissues and probe intercellularly with their stylet-like mouth parts to feed on nutrients translocating via phloem-sieve elements. Once the feeding structure is formed, the aphid can continue feeding at the same site for several days. Consequently, plants may have defense systems offering both quick and long-lasting responses. Thus, it is important to select an appropriate time point to profile the genes responsive to greenbugs. Moran and Thompson (2001) showed that a majority of aphid-induced genes, including genes which induced systemic defenses, peaked at 3 days post-infestation (dpi) in Arabidopsis. We therefore analyzed the gene expression in sorghum plants at 3 dpi with greenbugs. As a consequence of the difference in sampling time and comparative analyses, the profiles obtained in this study have a wide coverage of differentially expressed genes, especially these late-responsive genes, when compared with the other profiles constructed using greenbug-induced sorghum seedlings collected at 2 dpi (Zhu-Salzman et al. 2004).

In our data, a portion of the genes was identified to be regulated via SA- and JA-dependent signal cascades. This supports a paradigm that phloem-feeding elicits intermediary responses between wounding and pathogen infection (Moran and Thompson 2001). During phloem-feeding, aphids secrete saliva for multi purpose, including lubrication of stylets, optimization of redox conditions in plants, and prevention of plant defense responses (Miles 1999; Moran et al. 2002). Plants developed elaborate defense systems to confront these elusive challenges by aphids. They recognize components in aphid saliva that elicits reinforcement of plant defense responses (Zhu-Salzman et al. 2005). In addition, plants take warning from perceiving elicitors released from greenbug feeding sites, which in turn triggers the onset of plant defense responses (Schilmiller and Howe 2005). Binding of the elicitor systemin to the receptor SR160 activates phospholiapse, leading to the release of linolenic acid, which is a precursor of JA (Ryan and Pearce 2003). JA synthesized from linolenic acid is strongly involved in the induction of defense responses against insect feeding, mechanical wounding, and pathogen attack (Seo et al. 2001). Likewise, SA controls defense signaling in response to pathogen attack in plants. SA plays a pivotal role in regulation of local- and systemic-defenses, including induction of HR and SAR, as well as expression of pathogenesis-related (*PR*) genes (Durner et al. 1997). In our profiles, several genes elicited by SA and JA were identified to encode diverse proteins, including CPI, polyphenol oxidase, glucanase, catalase, ankyrin, cytochrome P450 monooxygenase, glutathione-S-transferase, and stearoyl-acyl carrier protein desaturase. Stearoyl-acyl carrier protein desaturase (S-ACP-DES) plays a key role in JA- and SA-dependent defense responses (Kachroo et al. 2004). S-ACP-DES converts stearic acid (18:0) to oleic acid (18:1). This conversion is a key step in maintaining the level of unsaturated fatty acids, leading to the activation of JA-mediated defense responses and repression of SA signaling cascade (Kachroo et al. 2003). The differential expression of the *S-ACP-DES* gene implies that interactions occurred between JA and SA during elicitation of sorghum defense responses against greenbug feeding.

For a deeper insight into the defense mechanisms of sorghum against greenbug feeding, two different microarray analyses were designed and performed. Unlike the first expectation, patterns of gene regulation in the two microarray analyses showed extensive dissimilarities. The dissimilarities were probably attributed to a severe difference in the level of damage inflicted on the seedlings of Mi and Ti at the time of harvesting, as well as differences in the genotypes between the two sorghum lines. After 72 h of greenbug infestation, Mi maintained healthy green seedlings nearly equal to those from untreated control sorghum (Fig. 1a). In addition, a portion of greenbugs infested on Mi fell down to the ground and died with unidentified reasons. On the contrary, Ti showed severe wilting and widespread necrotic spots (Fig. 1b, c). The microarray analysis between Mi and Mni showed an overall upregulation of defense related genes in concurrence with up- and down regulation of oxidative burst related genes. The genes related to oxidative burst, encoding CAT, PX, and QR, quench H_2O_2 generation that leads to the induction of the defense responses in plants (Orozco-Cardenas et al. 2001). The up- and down regulation patterns of the oxidative burst-related genes imply that ROS accumulation and detoxification of ROS occurred simultaneously during greenbug feeding. The microarray analysis between Mi and Ti showed an overall down regulation of the *CAT, PX*, and *QR* genes with concurrent down regulation of several defense-related genes. The reason for down regulation of defense-related genes in spite of down regulation of oxidative burst-related genes remains uncertain, but we assume that ROS burst occurred intensely in Ti during the early stage of greenbug feeding. Therefore, levels of ROS remained high enough to induce defense-related genes before harvesting seedlings of Ti, even though scavenging of ROS has already begun. Strikingly, defense-related genes were verified to be upregulated in both Mi and Ti. For instance, our northern-blot analyses showed that the genes encoding beta-glucosidase (*Glu*) and beta glucanase (*BGL*) were much more highly induced in Ti than the expression levels of those genes in Mi (Fig. 6). The question remained about what factors caused Mi to

possess a strong resistant phenotype to greenbug, compared to a high susceptibility of Ti. Considering the results from both microarray analyses, reinforcement of cell wall presumably played a crucial part in conferring resistance to greenbugs in M627 line.

Reinforcement of cell walls is one of the major defense strategies employed by plants (Minorsky 2002). Two genes, *COMT* and *PRP*, were co-upregulated in both microarray analyses, and the other genes involved in cell wall fortification were also upregulated, respectively. In our profiles, genes related to cell wall fortification include cellulose synthase (*Ces*), glycosyl transferase (*GLT*), and pyrroline-5-carboxylate dehydrogenase (*P5CDH*). The *Ces* was upregulated by MeJA treatment on sorghum seedlings, and differentially regulated by fungal infection (Schenk et al. 2000; Salzman et al. 2005). GLT is known to play a key part in cellulose synthesis, and P5CDH is involved in the control of proline degradation (Holland et al. 2000; Deuschle et al. 2004). Strong induction of the *P5CDH* gene was observed in Ti from 12 h of greenbug infestation on the contrary to a noticeably minor induction at 24 h of greenbug infestation in Mi (Fig. 6). This supports the idea that cell wall fortification played a crucial part in a strong resistant phenotype in Mi against greenbug feeding. However, a previous study (Zhu-Salzman et al. 2004) showed the lack of cell wall fortification-related genes when using only a susceptible sorghum line challenged with greenbugs.

Here we presented the transcriptome profiles of sorghum genes in response to greenbug phloem-feeding and interpreted the regulation patterns of greenbug-responsive genes in sorghum. In addition, the putative functions of genes were identified and linked to plant metabolic processes to understand mechanisms of sorghum defense systems against greenbug phloem-feeding. Some of the transcriptome profiles were verified to be controlled by several molecular regulators, including SA, JA, ABA, auxin, and GA. A gene encoding AIP, which was co-upregulated in both microarray analyses, was profiled. Two other genes encoding GA-induced protein and another auxin-regulated protein were also differentially regulated in response to the greenbug feeding (Table 1). Precise roles of auxin and GA in defense events against greenbug phloem-feeding have remained elusive. Auxin homeostasis and maintenance of capturing auxin signaling are important in mounting defense responses (Mayda et al. 2000). GA is a well-known growth regulator, but its role in defense events is not clear. A previous study showed that a GA treatment enhanced the germination rate of chick pea seeds, which was repressed by salt stress by increasing amylase activity and starch translocation rate (Kaur et al. 1998). Interactions between plant and insect are extremely complex, thereby much remains to be studied. In particular, investigation in the field of interactions between phloem-feeding insects and plants has been much less exploited and remains to be explored in spite of recent progress. More studies are required to elucidate a detailed mechanism of inducing plant defense responses by phloem-feeding insects. Additionally, more efforts on the interpretation of complex interactions among molecular regulators will pave the way for understanding the control mechanisms of defense events in plants.

In conclusion, using a combination of cDNA subtraction and microarray analysis, sorghum genes responsive to greenbug phloem-feeding were profiled and identified. In total, 157 transcripts verified to be involved in defense responses against greenbugs were obtained. Amongst the profiles, several genes, including *Thi* and *Xa1*, were newly identified to be involved in defense reactions on phloem-feeding herbivory. In addition, the two molecular regulators, auxin and GA, were verified to be involved in the regulation of defense responses against greenbugs in sorghum. Lastly, cell wall fortification proved to be an important factor in determining assignment of resistance to greenbugs.

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References

Ahmadi A, Baker DA (2001) The effect of water stress on the activities of key regulatory enzymes of the sucrose to starch pathways in wheat. Plant Growth Regul 35:81–91

Aroca R, Irigoyen JJ, Sanchez-Diaz M (2003) Drought enhances maize chilling tolerance. II Photosynthetic traits and protective mechanisms against oxidative stress. Physiol Plant 117:540–549

Birkett MA, Campbell CAM, Chamberlain K, Guerrieri E, Hick AJ, Martin JL, Matthes M, Napier JA, Petterson J, Pickett JA, Poppy GM, Pow EM, Pye BJ, Smart LE, Wadhams LJ, Woodcock CM (2000) New roles for *cis*-jasmone as an insect semiochemical and in plant defense. Proc Natl Acad Sci USA 97:9329–9334

Botella MA, Xu Y, Prabha TN, Zhao Y, Narashimhan ML, Wilson KA, Nielsen SS, Bressan RA, Hasegawa PM (1996) Differential expression of soybean cysteine proteinase inhibitor genes during development and in response to wounding and methyl jasmonate. Plant Physiol 112:1201–1210

Dayakar BV, Lin HJ, Chen CH, Ger MJ, Lee BH, Pai CH, Chow D, Huang HE, Hwang SY, Chung MC, Feng TY (2003) Ferredoxin from sweet pepper (*Capsicum annuum* L.) intensifying harpin$_{pss}$-mediated hypersensitive responses shows an enhanced production of active oxygen species (AOS). Plant Mol Biol 51:913–924

Deuschle K, Funck D, Forlani G, Stransky H, Biehl A, Leister D, van der Graaff E, Kunze R, Frommer WB (2004) The role of Δ^1-pyrroline-5-carboxylate dehydrogenase in proline degradation. Plant Cell 16:3413–3425

Dmitriev AP (2003) Signal molecules for plant defense responses to biotic stress. Russ J Plant Physiol 50:417–425

Doares SH, Navaez-Vasquez J, Conconi A, Ryan CA (1995) Salicylic acid inhibits synthesis of proteinase inhibitors in tomato leaves induced by systemin and jasmonic acid. Plant Physiol 108:1741–1746

Durner J, Shah J, Klessig DF (1997) Salicylic acid and disease resistance in plants. Trends Plant Sci 2:266–274

Ecker JR (1995) The ethylene signal transduction pathway in plants. Science 268:667–675

Felton GW, Korth KL, Bi JL, Wesley SV, Huhman DV, Mathews MC, Murphy JB, Lamb C, Dixon RA (1999) Inverse relationship between systemic resistance of plants to microorganisms and to insect herbivory. Curr Biol 9:317–320

Girousse C, Moulia B, Silk W, Bonnemain JL (2005) Aphid infestation causes different changes in carbon and nitrogen allocation in alfalfa stems as well as different inhibitions of longitudinal and radial expansion. Plant Physiol 137:1474–1484

Halitschke R, Schittko U, Porhnert G, Boland W, Baldwin IT (2001) Molecular interactions between the specialist herbivore *Manduca sexta* (Lepidoptera, Sphingidae) and its natural host *Nicotiana attenuata* III Fatty acid–amino acid conjugates in herbivore oral secretions are necessary and sufficient for herbivore-specific plant responses. Plant Physiol 125:711–717

Hammond-Kosack KE, Jones JDG (1996) Resistance gene-dependent plant defense responses. Plant Cell 8:1773–1791

Holland N, Holland D, Helentjaris T, Dhugga KS, Xoconostle-Cazares B, Delmer DP (2000) A comparative analysis of the plant cellulose synthase (CesA) gene family. Plant Physiol 123:1313–1323

Kachroo A, Lapchyk L, Fukushige H, Hildebrand D, Klessig D, Kachroo P (2003) Plastidial fatty acid signaling modulates salicylic acid- and jasmonic acid-mediated defense pathways in the Arabidopsis *ssi2* mutant. Plant Cell 15:2952–2965

Kachroo A, Venugopal SC, Lapchyk L, Falcone D, Hildebrand D, Kachroo P (2004) Oleic acid levels regulated by glycerolipid metabolism modulate defense gene expression in *Arabidopsis*. Proc Natl Acad Sci USA 101:5152–5157

Katsar CS, Paterson AH, Teetes GL, Peterson GC (2002) Molecular analysis sorghum resistance to the greenbug (Homoptera: Aphididae). J Econ Entomol 95:448–457

Kaur S, Gupta AK, Kaur N (1998) Gibberellin A_3 reverses the effect of salt stress in chick pea (*Cicer arietinun* L.) seedlings by enhancing amylase activity and mobilization of starch in cotyledons. Plant Growth Regul 26:85–90

Kawano T (2003) Roles of the reactive oxygen species-generating peroxidase reactions in plant defense and growth induction. Plant Cell Rep 21:829–837

Kessler A, Baldwin IT (2002) Plant responses to insect herbivory: The emerging molecular analysis. Annu Rev Plant Biol 53:299–328

Kobayashi I, Hakuno H (2003) Actin-related defense mechanism to reject penetration attempt by a non-pathogen is maintained in tobacco BY-2 cells. Planta 217:340–345

Li H, Shen JJ, Zheng ZL, Lin Y, Yang Z (2001) The ROP GTPase switch controls multiple developmental processes in Arabidopsis. Plant Physiol 126:670–684

Li L, Steffens JC (2002) Overexpression of polyphenol oxidase in transgenic tomato plants results in enhanced bacterial disease resistance. Planta 215:239–247

Lu H, Rate DN, Song JT, Greenberg JT (2003) ACD6, a novel ankyrin protein, is a regulator and an effector of salicylic acid signaling in the Arabidopsis defense response. Plant Cell 15:2408–2420

Majoul T, Bancel E, Tribol E, Hamida JB, Branlard G (2004) Proteomic analysis of the effect of heat stress on hexaploid wheat grain: characterization of heat-responsive proteins from non-prolamins fraction. Proteomics 4:505–513

Maleck K, Dietrich RA (1999) Defense on multiple fronts: how do plants cope with diverse enemies? Trends Plant Sci 4:215–219

Mano J, Torii Y, Hayashi SI, Takimoto K, Matsui K, Nakamura K, Inze D, Babiychuk E, Kushnir S, Asada K (2002) The NADPH: quinine oxidoreductase P1-ζ-crystallin in *Arabidopsis* catalyzes the α, β-hydrogenation of 2-alkenals: detoxification of the lipid peroxide-derived reactive aldehydes. Plant Cell Physiol 43:1445–1455

Mayda E, Marques C, Conejero V, Vera P (2000) Expression of a pathogen-induced gene can be mimicked by auxin insensitivity. Mol Plant Microbe Interact 13:23–31

Miles PW (1999) Aphid saliva. Biol Rev 74:41–85

Minorsky PV (2002) The wall becomes surmountable. Plant Physiol 128:345–353

Moran PJ, Thompson GA (2001) Molecular responses to aphid feeding in Arabidopsis in relation to plant defense pathways. Plant Physiol 125:1074–1085

Moran PJ, Cheng Y, Cassell JL, Thompson GA (2002) Gene expression profiling of *Arabidopsis thaliana* in compatible plant-aphid interactions. Arch Insect Biochem 51:182–203

Morreel K, Ralph J, Lu F, Goeminne G, Busson R, Herdewijn P, Goeman JL, Van der Eycken J, Boerjan W, Messens E (2004) Phenolic profiling of caffeic acid *O*-methyltransferase-deficient poplar reveals novel benzodioxane oligolignols. Plant Physiol 136:4023–4036

Narusaka Y, Narusaka M, Seki M, Umezawa T, Ishida J, Nakajima M, Enju A, Shinozaki K (2004) Crosstalk in the responses to abiotic and biotic stresses in *Arabidopsis*: analysis of gene expression in *cytochrome P450* gene superfamily by cDNA microarray. Plant Mol Biol 55:327–342

Oh BJ, Ko MK, Kostenyuk I, Shin BC, Kim KS (1999) Coexpression of a defensin gene and a thionin-like gene via different signal transduction pathways in pepper and *Colletotrichum gloeosporioides* interaction. Plant Mol Biol 41:313–319

Orozco-Cardenas ML, Narvaez-Vasquez, Ryan CA (2001) Hydrogen peroxide acts as a second messenger for the induction of defense genes in Tomato plants in response to wounding, systemin, and methyl jasmonate. Plant Cell 13:179–191

Pare PW, Tumlinson JH (1999) Plant volatiles as a defense against insect herbivores. Plant Physiol 121:325–331

Park JA, Cho SK, Kim JE, Chung HS, Hong JP, Hwang B, Hong CB, Kim WT (2003) Isolation of cDNAs differentially expressed in response to drought stress and characterization of the *Ca-LEAL1* gene encoding a new family of atypical LEA-like protein homologue in hot pepper (*Capsicum annum* L.cv. pukang). Plant Sci 165:471–481

Pechan T, Ye L, Chang YM, Mitra A, Lin L, Davis FM, Williams WP, Luthe DS (2000) A unique 33-kD cysteine proteinase accumulates in response to larvar feeding in maize genotypes resistant to fall armyworm and other Lepidoptera. Plant Cell 12:1031–1040

Pena-Cortes H, Albrecht T, Prat S, Weiler EW, Willmitzer L (1993) Aspirin prevents wound-induced gene expression in tomato leaves by blocking jasmonic acid biosynthesis. Planta 191:123–128

Penninckx IAMA, Thomma BPHJ, Buchala A, Metraux JP, Broekaert WF (1998) Concomitant activation of jasmonate and ethylene response pathways is required for induction of a plant defensin gene in Arabidopsis. Plant Cell 10:2103–2113

Pichersky E, Gershenzon J (2002) The formation and function of plant volatiles: perfumes for pollinator attraction and defense. Curr Opin Plant Biol 5:237–243

Powell G (2005) Intracellular salivation is the aphid activity associated with inoculation of non-persistently transmitted viruses. J Gen Virol 86:469–472

Reymond P, Farmer EE (1998) Jasmonate and salicylate as global signals for defense gene expression. Curr Opin Plant Biol 1:404–411

Riccardi F, Gazeau P, de Vienne D, Zivy M (1998) Protein changes in response to progressive water deficit in maize. Plant Physiol 117:1253–1263

Ryan CA (2000) The systemin signaling pathway: differential activation of plant defensive genes. Biochim Biophys Acta 1477:112–121

Ryan CA, Pearce G (2003) Systemin: a functionally defined family of peptide signals that regulate defensive genes in Solanaceae species. Proc Natl Acad Sci USA 100:14577–14580

Salzman RA, Brady JA, Finlayson SA, Buchanan CD, Summer EJ, Sun F, Klein PE, Klein RR, Pratt LH, Cordonnier-Pratt MM, Mullet JE (2005) Transcriptional profiling of sorghum induced by methyl jasmonate, salicylic acid, and aminocyclopropane carboxylic acid reveals cooperative regulation and novel gene responses. Plant Physiol 138:352–368

Seo HS, Song JT, Cheong JJ, Lee YH, Lee YW, Hwang IG, Lee JS, Choi YD (2001) Jasmonic acod carboxyl methyltransferase: a key enzyme for jasmonate-regulated plant responses. Proc Natl Acad Sci USA 98:4788–4793

Schenk PM, Kazan K, Wilson I, Anderson JP, Richmond T, Somerville SC, Manners JM (2000) Coordinated plant defense responses in Arabidopsis revealed by microarray analysis. Proc Natl Acad Sci USA 97:11655–11660

Schilmiller AL, Howe GA (2005) Systemic signaling in the wound response. Curr Opin Plant Biol 8:369–377

Shields JM, Pruitt K, McFall A, Shaub A, Der CJ (2000) Understanding Ras: 'it ain't over 'til it's over'. Trends Cell Biol 10:147–154

Shufran KA, Burd JD, Anstead JA, Lushal G (2000) Mitochondrial DNA sequence divergence among greenbug (Homoptera: Aphididae) biotypes: evidence for host-adapted races. Insect Mol Biol 9:179–184

Silvente S, Camas A, Lara M (2003) Molecular cloning of the cDNA encoding aspartate aminotransferase from bean root nodules and determination of its role in nodule nitrogen metabolism. J Exp Bot 54:1545–1551

Stone BS, Shufran RA, Wilde GE (2000) Life history of multiple clones of insecticide resistant and susceptible greenbug *Schizaphis graminum* (Homoptera: Aphididae). J Econ Entomol 93:971–974

Stotz HU, Kroymann J, Mitchell-Olds T (1999) Plant-insect interactions. Curr Opin Plant Biol 2:268–272

Ting Lee ML, Kuo FC, Whitmore GA, Sklar J (2000) Importance of replication in microarray gene expression studies: statistical methods and evidence from repetitive cDNA hybridizations. Proc Natl Acad Sci USA 97:9834–9839

Tuinstra MR, Wilde GE, Kriegshaauser T (2001) Genetic analysis of biotype I greenbug resistance in sorghum. Euphytica 121:87–91

Turner JG, Ellis C, Devoto A (2002) The jasmonate signal pathway. Plant Cell 14:S153–S164

Vignols F, Jose-Estanyol M, Caparros-Ruiz D, Rigau J, Puigdomenech P (1999) Involvement of a maize proline-rich protein in secondary cell wall formation as deduced from its specific mRNA localization. Plant Mol Biol 39:945–952

Voelckel C, Weiser WW, Baldwin IT (2004) An analysis of plant-aphid interactions by different microarray hybridization strategies. Mol Ecol 13:3187–3195

Walling LL (2000) The myriad plant responses to herbivores. J Plant Growth Regul 19:195–216

Wang KLC, Li H, Ecker JR (2002) Ethylene biosynthesis and signaling networks. Plant Cell 14:S131–S151

Watanabe T, Kitagawa H (2000) Photosynthesis and translocation of assimilates in rice plants following phloem feeding by the planthopper *Nilaparvata lugens* (Homoptera: Delphacidae). J Econ Entomol 93:1192–1198

Yoshimura S, Yamanuchi U, Katayose Y, Toki S, Wang ZX, Kono I, Kurata N, Yano M, Iwata N, Sasaki T (1998) Expression of Xa1, a bacterial blight-resistance gene in rice, is induced by bacterial inoculation. Proc Natl Acad Sci USA 95:1663–1668

Zhu-Salzman K, Salzman S, Ahn JE, Koiwa H (2004) Transcriptional regulation of sorghum defense determinants against a phloem-feeding aphid. Plant Physiol 134:420–431

Zhu-Salzman K, Bi JL, Liu TX (2005) Molecular strategies of plant defense and insect counter-defense. Insect Sci 12:3–15